Factors influencing predation on eggs and tadpoles of the endangered Green and Golden Bell Frog *Litoria aurea* by the introduced Plague Minnow *Gambusia holbrooki*

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ABSTRACT

The Green and Golden Bell Frog *Litoria aurea* has declined dramatically in New South Wales. One hypothesis suggests that this decline is largely due to predation by the Plague Minnow *Gambusia holbrooki*, which was introduced into Australia as part of efforts to control mosquitoes. Other frog species, such as Striped Marsh Frog *Limnodynastes peronii* and Peron's Tree Frog *Litoria peronii* have not apparently suffered from this predation to the same degree.

Laboratory experiments were undertaken in order to evaluate the extent to which predation on eggs, fry and tadpoles of *L. aurea* by *Gambusia* is influenced by the presence of rocks (as potential shelter), the presence of other frog species (as alternative food) and a number of variables that reflect the ratio of the number of prey available to the amount of food that would be required to satiate the fish. These experiments showed that the proportion of available *L. aurea* eggs or tadpoles eaten increased with both decreases in the number of prey available and increases in the satiation requirements of the fish. Less expected, however, were the results that the extent of predation on *L. aurea* did not depend on whether rocks were present, nor on whether other frog species were present. Similarly, predation on both the *Lim. peronii* and the *L. peronii* was not influenced by whether these species were presented to the fish on their own or in the presence of one or other of the other two frog species.

Gambusia was observed to employ different predatory strategies when attacking tadpoles of different sizes. Tadpoles were most often attacked from the rear, usually by a school of fish. Larger tadpoles were rarely eaten after being killed. Smaller tadpoles and fry were eaten mid-ventral region first, often only the viscera and yolk sac being consumed. *L. aurea* eggs were torn open and sometimes the yolk was consumed.

Preliminary experiments on the extent of predation on *L. aurea* eggs, fry and tadpoles by Empire Gudgeon *Hypseleotris compressa*, Firetailed Gudgeon *H. galii*, Pacific Blue-eye *Pseudomugil signifer* and Red-fin Perch *Perca fluviatilis* were also carried out. These experiments suggest that these fish will, if sufficiently hungry, eat the eggs, fry and tadpoles of *L. aurea* but that the extent of such predation may be generally less than with *Gambusia* under similar circumstances. Further research in this area is necessary before management implications are drawn.

ABSTRACT

The experimental studies of predation by *Gambusia* demonstrate that, under some circumstances, it is a voracious predator of eggs, fry and tadpoles of *L aurea*. This is consistent with the increase in abundance and distribution of *Gambusia* being a major factor in bringing about the decline of *L aurea* and indicates that steps should be taken to limit further spread of this fish into areas where *L aurea* is found. However, the few observations of *L aurea* apparently surviving and breeding in water bodies occupied with *Gambusia* suggest that it may be possible to develop management strategies that reduce any impact of *Gambusia* on this frog species in places where the fish is already present.

Key words: frog, fish, *Litoria aurea*, *Gambusia*, predation, feral, management, pest

INTRODUCTION

The Green and Golden Bell Frog *Litoria aurea* is presently considered endangered in New South Wales, having disappeared from over 90% of sites where it once occurred (White and Pyke 1996). It is now apparently extinct west of the Dividing Range, including the Australian Capital Territory (Osborne *et al.* 1996; White and Pyke 1996), and restricted to only about 30 coastal sites in New South Wales, at most of which the populations appear to be small (White and Pyke 1996). The species has apparently not declined to the same degree in Victoria (Gillespie 1996) and it does not occur elsewhere in Australia (White and Pyke 1996).

Predation by the introduced Plague Minnow *Gambusia holbrooki* (hereafter referred to as *Gambusia*) is considered to have been a major factor in the demise of *L. aurea* in New South Wales and the Australian Capital Territory (Mahony 1993, 1996; Harris 1995; Goldingay 1996; Morgan and Buttermer 1996; Osborne *et al.* 1996; Osborne and McElhinney 1996; White and Pyke 1996). This fish species occurs at many of the sites from which L. *aurea* has disappeared, breeding by *L. aurea* is generally restricted to water bodies that lack the fish, predation on *L. aurea* tadpoles is higher in ponds with *Gambusia* than in similar ponds without the species, and experiments have shown that *Gambusia* will readily eat *L. aurea* tadpoles (Morgan and Buttermer 1996; Pyke and White 1996; White and Pyke 1996). Other factors that have or may have contributed to the decline in L. aurea include habitat destruction, habitat modification, increased UV radiation and disease (Clancy 1996; Daly 1996; Goldingay 1996; Osborne *et al.* 1996; van de Mortel and Buttermer 1996; White and Pyke 1996).

Predation by *Gambusia* is also a potential threat to Victorian populations of *L. aurea* (Goldingay 1996). This fish is presently absent from much of East Gippsland where most Victorian populations of *L. aurea* occur (McDowell 1980; Gillespie 1996). However, populations of this fish have already penetrated most of Eastern Victoria, and there are no apparent impediments to further spread of the species through East Gippsland (White and Pyke, unpubl. obs.). The effects of such spread of *Gambusia* are likely to be devastating for Victorian populations of *L. aurea*.

There are a few sites where *L. aurea* and *Gambusia* co-exist and indeed some water bodies where *L. aurea* has apparently been able to breed despite the presence of *Gambusia* (White and Pyke 1996; van de Mortel and Goldingay 1998). Studies of these sites may increase our understanding of the relationship between these two species and, as a result, improve our abilities to conserve and manage this endangered frog (Goldingay 1996; van de Mortel and Goldingay 1998).

Litoria aurea may be able to co-exist and breed in the presence of *Gambusia* if the water bodies: (a) have a relatively low ratio of fish density to frog density; (b) have relatively more available shelter for tadpoles, thus enabling some to escape predation by the fish; (c) provide adequate alternative food to the fish, thus decreasing the tendency of the fish to prey on the eggs or tadpoles of the frog; or (d) differ in some other way.

Some frog species seem to have persisted better than *L. aurea* in the presence of *Gambusia*. At some sites, for example, where *L. aurea* has apparently disappeared in association with invasion by *Gambusia*, there remain populations of other frog species such as the Striped Marsh Frog *Limnodynastes peronii* and Peron's Tree Frog *Litoria peronii* (White and Pyke 1996; Pyke and

White 1996). One possible explanation for this is that *Gambusia* prefer to attack *L. aurea* than either of the other two species.

Observations suggest that other small predatory fish species may affect *L. aurea* in a similar fashion to *Gambusia* (A. White pers. obs). For example, at Greenacre (a suburb of Sydney) where *L. aurea* occurs (see White and Pyke 1996) tadpoles of this species have rarely been observed, but there are no *Gambusia* present. Instead there are high densities of Empire and Firetail Gudgeon (*Hypseleotris compressa* and *H. galii* respectively). *Litoria aurea* also occurs, but does not apparently breed successfully, at a pond at Hammondville (see White and Pyke 1996) where there are large numbers of Red-fin Perch *Perca fluviatilis* but no *Gambusia*. The Empire and Firetail Gudgeons are both predatory fish species that are similar in size to *Gambusia*, while the predatory Red-fin Perch is of a similar size when relatively young (McDowell 1980).

If these four fish species are significant predators of the eggs, fry or tadpoles of *L. aurea*, then their spread could contribute to further declines in this species. It is therefore important to investigate the nature and extent of any predation on *L. aurea* by these fish species.

The principal aim of this study was to conduct laboratory experiments to explore the above possible explanations for apparent coexistence of *L. aurea* and *Gambusia*. This was achieved through experimental manipulation of fish, eggs or tadpoles, availability of other food for the fish, and availability of shelter for tadpoles. A secondary aim was to determine whether the other fish species mentioned above will eat *L. aurea* eggs or tadpoles and to compare the extent of predation by these species with that by *Gambusia*.

METHODS

All experiments were carried out indoors at the home of the junior author (A.W.) over the spring/summer periods of 1994/95 and 1995/96. All trials were carried out using empty white plastic 2-litre icecream containers filled with 1.8 litres of aged and decanted tap water. Each experiment lasted two days and the water was not changed or aerated during this time. Water temperatures were measured but not regulated, and ranged between 17 and 22 C. In some of the experiments shelter for tadpoles was provided by the inclusion of 3-4 rocks, each roughly 5-10 cm in diameter. Rocks were chosen, in preference to alternatives such as vegetation, because they could not be moved or eaten by the animals during the experiments. They were positioned so that they afforded potential shelter between them as well as around them.

In each experiment, fish and either eggs or tadpoles were placed in the same container. The number of eggs or tadpoles remaining intact was recorded after one and two days. *Gambusia* were grouped according to size; small ranged from 1 to 1.5 cm in length, medium ranged from 1.5 to 2.5 cm, while large ranged from 2.5 to 4.0 cm. Tadpoles were also grouped according to size and developmental stages (Gosner 1960): fry were less than 48 hours old and had not developed past Stage 21; small ranged from 0.8 to 1.3 cm in length and varied from stage 21 to stage 25; medium ranged from 1.3 to 2.0 cm and varied in stage from 25 to 27; large were greater than 2.0 cm in length. In some experiments alternative food for the fish was made available by placing both commercial fish flakes and mosquito larvae in the containers before the tadpoles and fish were introduced. No individual tadpole or fish was used more than once across the range of experiments.

The variables that were considered in our experiments were the type and number of potential prey, the size of fish predator, the duration of any pre-experiment starvation period for the fish, whether or not additional food was provided for the fish, and whether or not rocks were present (Tables 1a and 1b). Potential prey included *L. aurea* in all experiments except Expt 9 in which potential prey were *Lim. peronii* and *L. peronii* (Tables 1a and 1b). Predators were *Gambusia* in Experiments 1-10. In Experiments 11-14 the predators were either Empire Gudgeon, Firetail Gudgeon, Red-fin Perch or Pacific Blue-eye. Potential prey were either eggs, fry or tadpoles of various sizes (see above). Fish predators were categorised as small, medium or large in size (see above). Prior to some of the experiments the fish were starved for various lengths of time. In experiment #10, rocks were present as potential sources of shelter for tadpoles.

There were 10 replicates for each treatment or experimental combination involving predation by *Gambusia*. In Experiment 1, for example, there were four treatments (i.e., two initial numbers x whether or not additional food provided) and therefore 40 aquaria used. For the experiments involving the other fish species, each treatment combination was implemented just once.

There were no treatments in which fish predation was absent. This should not, however, affect our analysis and conclusions, because all comparisons and conclusions were based on different predation circumstances, and tadpole mortality over such brief periods as we studied is usually negligible in the absence of predation (White, pers. obs.).

The results of the experiments involving predation by *Gambusia* were analysed using multiway Analysis of Variance (ANOVA). In cases where significant interactions occurred separate lower-order ANOVAs were carried out for each level of one or more of the experimental factors. In most cases the dependent variables were the number of eggs, fry or tadpoles remaining after 24 or 48 h. In a few cases, where the initial number was itself a factor, the proportions remaining after 24 and 48 h were essentially the dependent variables, but the analysis was carried out on the arcsine transformations of these proportions (see Tables 3, 5 and 8). Any significant results for these proportions are presented in terms of the untransformed proportion (see Tables 3, 5 and 8). Because about 60 statistical tests were carried out before significant interactions were dealt with (see below) we adopted a Bonferroni correction factor and used a threshold probability of 0.001 (i.e., 0.05/60 to three decimal places) for assessing the significance of each comparison (see below).

Because of the lack of replication for the experiments involving the other fish species and the fact that the presence/absence of additional food was a variable in all cases, we analysed the results of these experiments after first combining the results from the four species and ignoring differences in initial type and number of prey. We analysed the results using the Normal Approximation to the Binomial (Sokal and Rohlf 1981).

Observations were also made with regard to the nature and extent of damage done to the eggs, fry and tadpoles as well as the attack methods used by *Gambusia* and the other fish to deal with tadpoles of different sizes.

RESULTS

Three-way interactions among the independent variables were not significant for any of the relevant experiments (i.e., Experiments 3, 4 and 5). These interactions were therefore omitted from the tables of results and are not discussed further. There were significant pairwise interactions among the independent variables for Experiments 1-5 (see Tables 3-6). Further analysis was therefore necessary for each category of at least one of the independent variables as described above (see Tables 3-6). In all cases, however, no more than two of the three possible interactions were significant (see Tables 3-6). Consequently, it was not necessary to consider more than one of the independent variables in this manner (see Tables 3-6).

The results of the experiments involving predation by *Gambusia* can be summarised as follows (see Tables 3-9 for details):

1. *Gambusia* eat eggs, fry and tadpoles of *L. aurea*;
2. The proportion of fry or tadpoles of *L. aurea* eaten by *Gambusia* increases with

   - increases in the number (and hence density) of fish (see Expt 5; Table 6)

   - decreases in the number (and hence density) of tadpoles (see Expt 4; Table 5)

   - increases in fish starvation (see Expt. 6; Table 7)

   - decreases in supply of other fish food, which the *Gambusia* ate when available (see Expts. 1 to 6; Tables 3 to 7)
3. *Gambusia* eat more fry and small tadpoles of *L. aurea* than either eggs or larger tadpoles (see Expts 1,2,3 and 5; Tables 3, 4 and 6)
4. The number of *L. aurea* tadpoles eaten was not affected by the presence of rocks (see Expt. 10; Table 9)
5. There were no differences in the extent of predation on the three frog species when presented to the fish alone versus being simultaneously presented in pairs of species (see Expts. 7, 8 and 9; Table 8)

The results of the experiments involving predation by the other fish species can be summarised as follows (See Table 2):

6. The overall levels of predation were extremely small, except for when the fish were left with their prey for 48 h without additional food (Table 2).
7. The highest level of predation observed for these other fish species was lower than observed levels for *Gambusia* predation (i.e., % survival of 84% when these other fish are left without additional food for 48 h vs. % survival ranging from 0% to about 90% when *Gambusia* are left for 48 h; see Tables 2-7)

Though not quantified, additional observations of interactions between tadpoles and fish, indicated that attacks by *Gambusia* against *L. aurea* eggs and tadpoles generally have the following features:

(a) A fish will often repeatedly attack a tadpole without initially killing it;

(b) Fish will often group when attacking tadpoles;

(c) Fish generally attack tadpoles from behind, either biting off the tail fin or muscular rod, or peeling off strips of skin. In either case a tadpole is immobilised or has limited ability to swim.

(d) Fish feeding behaviour generally depends on the type and size of prey and sometimes on the size of the fish. Eggs are normally ruptured, but only occasionally is the yolk eaten. Small to medium tadpoles were generally eviscerated after being immobilised; occasionally their body walls were also eaten. Large tadpoles were usually killed but not eaten. Relatively large fish will sometimes herd tadpoles, continually buffeting them, before removing their tails.

Table 1a. Design of experiments involving predation by *Gambusia* on eggs and tadpoles of *L. aurea*

Expt	Main effects being tested for	Number/Type[1] (E=eggs; F=fry; S=small/M= medium/ L=large tadpoles)	Fishsize (S=small; M=medium; L=large)	Starv. Period	Whether additional food	Whether rocks present
1	Initial number; Whether additional food	5E or 20E	5M	24h	Y, N	N
2	As above	5F or 20F	5M	24h	Y, N	N
3	Fry vs tadpoles; fish size; Whether additional food	5F or 5M	11S,5.5M or 2.5L	0	Y, N	N
4	Fry vs tadpoles; Initial number; Whether additional food	2F, 5F, 10F, 2M, 5M or 10M	5M	0	Y, N	N
5	Tadpole size; Fish number; Whether additional food	5S, 5M or 5L	2M, 5M or 10M	0	Y, N	N
6	Fish starvation period	5F	5M	0,24h,48h,72h	N	N
7	*L. aurea* vs *Lim. peronii*	*L. aurea*= 10M, 5M or 0 and *Lim. peronii*= 0, 5M or 10M	5M	24h	N	N
8	*L. aurea* vs *L. peronii*	*L. aurea*=10F, 5F or 0 and *L. peronii*=0, 5F or 10F	5M	24h	N	N
9	*L. peronii* vs *Lim. peronii*	*L. peronii*=10M, 5M or 0 and *Lim. peronii*=0 ,5M,10M	5M	24h	N	N
10	Fry vs tadpoles; Whether rocks present	5F or 5M	10M	0	N	Y/N

Notes: 1. All *L. aurea,* except experiments 7,8 and 9

Table 1b. Design of experiments involving predation by other fish species on eggs and tadpoles of *L. aurea*

Expt	Main effects being tested	Fish species	Number of fish	Prey Number/Type	Whether additional food
11	Eggs vs fry vs tadpoles; Whether additional food	Empire gudgeon (*Hypseleotris compressa*)	5	20E, 20F, 10S	Y/N
12	Whether additional food	Fire-tailed Gudgeon (*Hypseleotris galii*)	5	10S	Y/N
13	Whether additional food	Pacific Blue-eye (*Pseudomugil signifer*)	3	20F	Y/N
14	Eggs vs fry vs tadpoles; Whether additional food	Red-fin Perch (*Perca fluviatilis*)	5	20E, 20F, 10S	Y/N

Table 2. Results of experiments involving predation by species of fish other than *Gambusia* (Combined initial number of prey=130 for each cell in Table)

Whether additional food	Proportion remaining after 24h	Proportion remaining after 48h
N	0.97	0.84
Y	1.00	0.98
Probability difference	>0.05	<0.001

Table 3. Statistics for expts 1 and 2. Probabities are presented for each independent variable and for pairwise interactions of these variables, with significant effects indicated with an asterix (*). Where the effect of an independent variable is significant but any interaction with this variable is not significant, the mean values for the dependent variable for each level of the independent variable are given in parentheses.

Dep.Var.	Indept.Var.(X1)	Indept.Var. (X2)	Prob X1	Prob X2	Prob X1 x X2
# eggs or fry left after 24h	Whether addit. food (N/Y)	Eggs vs. Fry	0.958	0.000* (4.8 vs 2.6)	0.315
# eggs or fry left after 48h	"	"	0.565	0.000* (4.3 vs 1.3)	0.226
Prop. eggs or fry left after 24h	Initial number (5 or 20)	Eggs vs. Fry	0.210	0.000* (0.91 vs 0.68)	0.015
Prop. eggs or fry left after 48h	"	"	0.314	0.000*	0.000*
Prop. eggs left after 48h	"	Eggs only	0.044		
Prop. fry left after 48h	"	Fry only	0.000* (0.20 vs 0.58)		

Table 4. Statistics for Expt 3. Probabilities are presented for each independent variable and for pairwise interactions of these variables, with significant effects indicated with an asterix (*). Where the effect of an independent variable is significant but any interaction with this variable is not significant, the mean values for the dependent variable for each level of the independent variable are given in parentheses

Dep.Var.	Indep.Var. X1	Indep.Var. X2	Indep.Var. X3	Prob X1	Prob X2	Prob X3	Prob X1 x X2	Prob X1 x X3	Prob X2 x X3
# left after 24h	Fish size	Type (Fry vs med tads)	Whether addit. Food	0.091	0.000*	0.000*	0.001*	0.951	0.656
# left after 48h	"	"	"	0.002	0.000*	0.004	0.000*	0.074	0.075
# left after 24h	"	Fry only	"	0.005		0.019		0.736	
# left after 48h	"	"	"	0.000*		0.488		0.490	
# left after 24h	"	Med tads only	"	0.129		0.001* (4.8 vs 4.3)		0.129	
# left after 48h	"	"	"	0.165		0.000*		0.000*	
# left after 48h	Small fish only	Med tads only				0.331			
	Medium fish only	"				0.000* (4.5 vs 3.0)			
	Large fish only	"				0.009			

Table 5. Statistics for Expt 4. Probabilities are presented for each independent variable and for pairwise interactions of these variables, with significant effects indicated with an asterix (*). Where the effect of an independent variable is significant but any interaction with this variable is not significant, the mean values for the dependent variable for each level of the independent variable are given in parentheses

Dep. Var.	Indep. Var. X1	Indep. Var. X2	Indep. Var. X3	Prob X1	Prob X2	Prob X3	Prob X1 x X2	Prob X1 x X3	Prob X2 x X3
Prop. Left after 24h	Initial number	Whether addit. Food	Type (Fry vs med tads)	0.208	0.027	0.437	0.631	0.000*	0.057
Prop. Left after 48h	"	"		0.002	0.000*	0.692	0.682	0.000*	0.008
Prop left after 24h	"	"	Fry only	0.013	0.003		0.933		
Prop left after 48h	"	"	"	0.387	0.001* (0.68 vs 0.39)		0.883		
Prop left after 24h	"	"	Med tads only	0.000* (0.48, 0.87 and 0.91)	0.830		0.278		
Prop left after 48h	"	"	"	0.000* (0.20, 0.66 and 0.78)	0.292		0.300		

Table 6. Statistics for Expt 5. Probabilities are presented for each independent variable and for pairwise interactions of these variables, with significant effects indicated with an asterix (*). Where the effect of an independent variable is significant but any interaction with this variable is not significant, the mean values for the dependent variable for each level of the independent variable are given in parentheses

Dep. Var.	Indep. Var. X1	Indep. Var. X2	Indep. Var. X3	Prob X1	Prob X2	Prob X3	Prob X1 x X2	Prob X1 x X3	Prob X2 x X3
# left after 24h	Tadpole size (small, med, big)	No. Fish (2, 5, 10)	Whether addit. Food	0.000* (1.95, 4.20 & 4.25)	0.000* (4.23, 3.60 & 2.55)	0.009	0.053	0.292	0.032
# left after 48h	"	"	"	0.000*	0.000*	0.001*	0.043	0.001*	0.331
# left after 48h	Small tads only	"	"		0.000* (2.10, 0.75 & 0.25)	0.165			0.273
# left after 48h	Med tads only	"	"		0.000* (4.10, 3.45 & 2.45)	0.000* (3.90 vs 2.77)			0.187
# left after 48h	Big tads only	"	"		0.000* (4.70, 3.50 & 2.10)	0.773			0.919

Table 7. Statistics for Expt 6. Probabilities are presented and marked with an asterix (*) where significant. Where the effect of the independent variable is significant the mean values for the dependent variable for each level of the independent variable are given in parentheses

Dependent Variable	Independent Variable	Probabilities
# left after 24h	Fish starvation period (0, 24h, 48h, 72h)	0.000* (2.70, 2.50, 1.20 and 0.30)
# left after 48h	"	0.001* (1.50, 0.70, 0.30 and 0.00)

Table 8. Statistics for Expts 7, 8 and 9. Probabilites are presented. None are significant.

Dependent Variable	Independent Variable	Probabilities
Prop. *L. aurea* left after 24h	Species (only *L. aurea, L. aurea+Lim. peronii*)	0.592
Prop. *L. aurea* left after 48h	"	0.065
Prop. *Lim. peronii* left after 24h	Species (only *Lim. peronii, Lim. peronii+L. aurea*)	0.009
Prop. *Lim. peronii* left after 48h	"	0.021
Prop. *L. aurea* left after 24h	Species (only *L. aurea, L. aurea+L. peronii*)	0.077
Prop. *L. aurea* left after 48h	"	0.155
Prop. *L. peronii* left after 24h	Species (only *L. peronii, L. peronii+L. aurea*)	0.016
Prop. *L. peronii* left after 48h	"	0.168
Prop. *Lim. peronii* left after 24h	Species (only *Lim. peronii, Lim. peronii+L. peronii*)	0.864
Prop. *Lim. peronii* left after 48h	"	0.524
Prop. *L. peronii* left after 24h	Species (only *L. peronii, L. peronii+Lim. peronii*)	0.548
Prop. *L. peronii* left after 48h	"	0.442

Table 9. Statistics for Expt 10. Probabilities are presented for each independent variable and for pairwise interactions of these variables, with significant effects indicated with an asterix (*). Where the effect of an independent variable is significant but any interaction with this variable is not significant, the mean values for the dependent variable for each level of the independent variable are given in parentheses

Dep. Var.	Indept. Var. X1	Indept. Var. X2	Prob X1	Prob X2	Prob X1 x X2
# left after 24h	Whether rocks present	Type (Fry vs Med tads)	0.570	0.001* (2.25 vs 3.45)	0.160
# left after 48h	"	"	0.620	0.000* (1.10 vs 2.75)	0.075

DISCUSSION

Our experiments demonstrate that not only do *Gambusia* attack and feed on *L. aurea* eggs, fry and tadpoles but that the level of this predation (as measured in terms of numbers killed) was determined by the amount of food that would probably be required to satiate the fish (i.e. their hunger level) relative to the available biomass of prey. With a constant number of prey items available, the total fish satiation requirements for eggs, fry or tadpoles should increase with increases in fish number or the extent to which the fish were starved beforehand and with decreases in the availability of alternative food. The observed numbers of eggs, fry and tadpoles killed were consistent with these trends. Also, with decreases in the initial number of tadpoles, it apparently took proportionately more to satisfy the appetites of the fish.

These apparent effects, if measured in biomass eaten rather than numbers killed, would probably be more pronounced. The habit of *Gambusia* killing but not eating its prey is more likely to occur with decreasing hunger levels of the fish, and consequently, as discussed above, with decreasing biomass eaten. Hence the proportion of killed prey actually eaten probably increased with increases in biomass killed. This would accentuate the above effects.

It is not known why *Gambusia* sometimes killed their prey without eating all of it. It is possible that they were selecting preferred parts of their prey and were satisfied with just the most-preferred parts when they were not very hungry. It is also possible that the tadpoles are partially unpalatable.

Predation of fry and small tadpoles of *L. aurea* was greater than that of eggs. This could indicate a preference by *Gambusia* for fry and small tadpoles, or it could be due to other factors. It is possible, for example, that the fish do not perceive stationary eggs as potential food to the same degree as fry or tadpoles. This is the more likely explanation for the lower predation on the eggs, as the *Gambusia* would attack fry that were wiggling their tails while suspended at the water surface but would rarely attack stationary fry even when they passed quite close to them.

That the presence of rocks (shelter) did not influence the level of predation on *L. aurea* tadpoles suggests that rocks alone do not, in our relatively small containers, provide sufficient places for tadpoles to escape predatory fish. It is not possible, however, to extrapolate from this result in terms of the general importance of shelter in influencing levels of fish predation on *L. aurea* tadpoles. Other kinds of shelter, such as aquatic plants, could be important as shelter, either on their own or in combination with rocks, although the presence of pond weed (*Egeria densa*) did not apparently influence the level of *Gambusia* predation on *L. aurea* tadpoles in the experiments of Morgan and Buttemer (1996). Since *L. aurea* tadpoles spend most of their time at mid-water levels rather than at the water surface or on the bottom of a pond (White unpubl. obs.), it is possible that shelter must be available at such mid-levels to be effective.

The lack of any differences in levels of predation on the different frog species when simultaneously available as potential prey suggests that *Gambusia* have no feeding preference among fry or tadpoles of the three frog species considered. This further suggests that the *Lim. peronii* and *L. peronii* may escape *Gambusia* predation more than *L. aurea* by virtue of some aspects of their behaviour rather than because they are less preferred as prey. The habit of *Lim. peronii* tadpoles of swimming near the bottom of the water column (Martin and Littlejohn 1982) might, for example, reduce their vulnerability to predation by *Gambusia*. Further investigation is necessary in order to explore this and other possibilities.

A consistent conclusion from the various experimental studies of predation by *Gambusia* is that, under some circumstances, *Gambusia* is a voracious predator on the eggs, fry and tadpoles of *L. aurea* and other Australian frog species (this study; Webb 1994; Morgan and Buttemer 1996; Webb and Joss 1997). This supports the frequently-made assertion that the increase in the distribution and abundance of *Gambusia* has been a major factor in the decline of *L. aurea* and other frog species (Mahony 1993, 1996; Webb 1994; Harris 1995; White and Pyke 1996; Morgan and Buttermer 1996; Osborne *et al.* 1996; Osborne and McElhinney 1996). It also supports the recent listing of *Gambusia* as a "threatening process" under the New South Wales *Threatened Species Conservation Act 1995*.

However, the relative importance of *Gambusia* predation versus other factors in causing the decline of *L. aurea* remains unclear (Goldingay 1996). Both the decline of the species in places where *Gambusia* is absent and the ability of the species to survive and breed in water bodies where *Gambusia* is present (White and Pyke 1996) serve to highlight the likely importance of other factors. Further research on *Gambusia* and other possible factors is warranted.

The above results and observations suggest that strategies to promote the protection and recovery of *L. aurea* will include:

1. methods to reduce the likelihood of further spread of *Gambusia* into areas where it is presently absent;
2. methods to reduce the densities of *Gambusia* in places where it occurs;
3. methods (such as provision of places where tadpoles can shelter and avoid predation) to reduce the impacts of *Gambusia* in places where *L. aurea* either already occurs or could be introduced;
4. provision of *Gambusia*-free breeding sites.

Litoria aurea is apparently vulnerable to predation by other small predatory fish species besides *Gambusia*. However, our preliminary experiments suggest that these fish may only be significant predators of *L. aurea* when they are particularly hungry and that even then, their appetite for eggs and tadpoles of *L. aurea* may be lower than that for *Gambusia*. Hence, if control of mosquito larvae is considered essential and if some or all of these other fish species are found to be significant predators of mosquito larvae, they may be preferable species to use for control of mosquitoes than *Gambusia*. It should be stressed, however, that our experiments involving these other fish species require further repetition and development before such a conclusion would be warranted.

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